THE EFFECT OF THE INJECTION OF BOVINE BILE INTO RABBITS.*

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Following the injection of bovine bile into rabbits in order to see if any arterial lesions were produced, certain results were met which seemed worthy of reporting as a preliminary note.

Most of the experimental work reported upon bile injections has been in regard to its toxicity and its effect upon the circulation. Among the American workers may be mentioned Meltzer and Salant, King and Stewart, and Bunting and Brown. In the article by King and Stewart are many references to foreign authors. Few of the writers mention any lesions in the parenchymatous organs. In human cases with marked jaundice, pathologists emphasize the presence of abnormal amounts of fat in the different cells. Bunting and Brown mention degenerative lesions in the different organs and lesions of the peritoneal cavity, especially about the pancreas following the escape of bile or a single injection of rabbit's bile into the rabbit's peritoneal cavity.

In this work it was planned to inject bovine bile intravenously into rabbits a variable number of times and then examine the larger arteries and those in the organs for degenerative or other lesions. As the bile injured the ear veins so that repeated intravenous injections were impossible, subcutaneous and intraperitoneal injections were also used. The bovine bile was obtained from a slaughter house, sterilized by steam in the autoclave and kept in a cold room just above freezing temperature. As the flask containing the bile from the first animal broke during the experiments, bile from a second animal was collected and preserved in a similar

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manner. The results obtained from the two biles were different and therefore will be reported in separate groups.

The toxicity of the first bile towards rabbits was unfortunately not tested. With this bile three good sized rabbits were injected as follows:

Rabbit No. 1: 2-14-12, 2 cc. subcu.; 2-15-12, 2.5 cc. ear vein; 2-19-12, 4 cc. subcu.; 2-22-12, found dead and autopsied.

Rabbit No. 3: 2-14-12, 1 cc. ear vein; 2-15-12, 1 cc. ear vein; 2-19-12, 4 cc. subcu.; 2-28-12, killed and autopsied.

Rabbit No. 4: 2-21-12, 3.25 cc. ear vein; 20-27-12, killed instantly by intravenous injection of 4 cc. of second bile; autopsied.

Rabbit No. 1 of this series apparently died as a result of infection with a microörganism. The spleen at autopsy was tremendously enlarged and inflammatory lesions with bacteria present were found in many organs. Therefore it is impossible to say whether or not the degenerative lesions found in this animal were due to the bile injections. In all the animals injected subcutaneously a marked reaction was present at the site of inoculation. The heart, lungs, liver, spleen, adrenals, kidneys, and aorta were studied histologically after staining Zenker fixed tissue with eosin and methylene blue, and formalin fixed tissue with Scharlach R.

Except for the lesions in No. 1, presumably due to the infection, no lesions were demonstrable by the eosin and methylene blue stain in the organs or vessels which could be attributed to the action of the bile. In the sections stained by Scharlach R an increased amount of fat over what was present in several control rabbits was found in the heart muscle fibers and irregularly distributed throughout the liver cells in all three animals. In addition Rabbit No. 1 showed fat in the necrotic areas in the spleen. The vessels throughout were free from fat. In the kidneys certain tubules showed some fat in both the control rabbits and experimental animals.

It seemed reasonable to think that there might be some relation between the fatty changes in the heart and liver and the bile injections even if one animal had died from an acute

infection and the other two had been killed six and nine days after the last injection, especially as Bunting and Brown had found degenerative lesions after short exposure of less than twenty-four hours to bile toxins. Therefore, a second series of animals were injected a greater number of times and killed and autopsied soon after the last injection rather than waiting several days.

In this series all the injections were made with the second bile except the first injection into Rabbit No. 2. This second bile at the beginning and end of the experiments would cause convulsions and instant death to rabbits weighing about one thousand seven hundred grams upon injection of four cubic centimeters intravenously. Twelve cubic centimeters injected intraperitoneally in the afternoon proved fatal to a rabbit during the night.

Four rabbits were injected as follows:

Rabbit No. 2: 2–19–12, 4 cc. 1st bile ear vein; 2–27–12, 4 cc. 2d bile ear vein; 3–1–12, 1.5 cc. 2d bile ear vein; 3–4–12, 4 cc. 2d bile subcu.; 3–5–12, 4 cc. 2d bile intraperitoneal; 3–6–12, 3.5 cc. 2d bile intraperitoneal; 3–7–12, 4 cc. 2d bile subcu.; 3–11–12, 3 cc. 2d bile intraperitoneal; 3–14–12, 4 cc. 2d bile intraperitoneal; 3–18–12, 4 cc. same; 3–19–12, 2 cc. same; 3–20–12, 4 cc. same; 3–21–12, 4 cc. same; 3–22–12, 4 cc. same; 3–25–12, 4 cc. same; 3–26–12, 3.5 cc. same; 3–27–12, 4 cc. same; 3–28–12, 3 cc. same; 3–29–12, 4 cc. same; 3–30–12, killed and autopsied.

Rabbit No. 5: 2–28–12, 1 cc. 2d bile ear vein; 3–1–12, 1 cc. 2d bile ear; 3–4–12, 1 cc. 2d bile ear; 3–5–12, 2 cc. 2d bile intraperitoneal; 3–6–12, 2 cc. 2d bile intraperitoneal; 3–7–12, 4 cc. 2d bile subcu.; 3–8–12, killed and autopsied.

Rabbit No. 6: 2–28–12, 1 cc. 2d bile ear vein; 3–1–12, 1 cc. 2d bile ear; 3–4–12, 1 cc. 2d bile ear; 3–5–12, 1 cc. 2d bile intraperitoneal; 3–6–12, 1 cc. 2d bile intraperitoneal; 3–7–12, 3 cc. 2d bile subcu.; 3–8–12, killed and autopsied.

Rabbit No. 7: 3–29–12, 12 cc. 2d bile intraperitoneal; 3–30–12, found dead and autopsied.

No peritoneal lesions were seen except in No. 2, which received so many injections. In this animal there were many adhesions and pockets of pus in the peritoneal cavity, but in view of the many chances for infection and in view of

the absence of lesions in the other animals it was felt that these were probably not due to the bile.

The same organs were preserved and the same staining methods used as in the former series. In none of these four animals were any lesions found in the sections stained with eosin and methylene blue which could be attributed to the bile. In the sections stained for fat no excess was found in any organs over that found in the controls except a few scattered areas in the liver cells of No. 7 which received the least amount of bile. There were no degenerative lesions seen in any of the arteries. Therefore this second bile, which was toxic for the rabbit, did not produce any lesions demonstrable by the methods used, although the experiences with the first bile and Bunting and Brown's experiences with rabbits' bile made it seem probable that some lesions would occur.

These few experiments show that bovine bile is not as toxic towards rabbits nor as destructive towards rabbits' tissue as rabbits' bile has been reported to be. They suggest that bile from two animals of the same species may differ in its toxicity towards another animal. Further study upon the action of different kinds of bile towards the organs and tissue of animals would be of interest.

REFERENCES.

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3. Bunting and Brown. Jour. Exp. Med., xiv, 1911.